

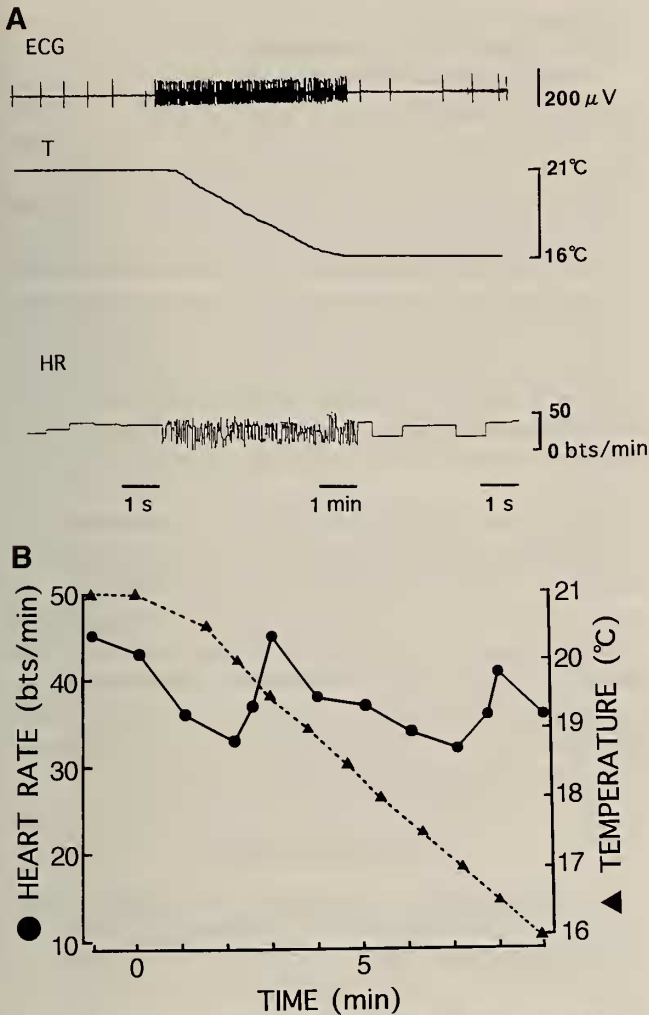


FIG. 2. Effects of cooling on the *in vivo* heart rate of the lobster in winter (14–2–1992). A, a simultaneous recording of ECG, ambient temperature (T) and heart rate (HR) displayed with fast and slow speeds. B, a graphic presentation of the heart rate change with decreasing temperature. Means of ten beats are successively plotted with the temperature. The data are from the same videotape recording shown in A.

always show a linear change with the decreasing temperature from 20 to 16°C. With rewarming, the heart rate returned and reached to a level of 5–10 bts/min higher than the initial rate before cooling (not shown in figure).

Correlation between the heart rate and temperature was analyzed statistically using the data shown in Table 1. In the period from spring to fall, the mean heart rates (68–99.5 bts/min) regressed on a positive linear line ($Y = 6.43X - 27.66$, $r = 0.99$) within the temperature range of 15–20°C (Fig. 3a). In contrast, the mean heart rates in winter showed a lower value (28–45.5 bts/min) and regressed on another positive line ($Y = 3.26X - 26.38$, $r = 0.88$) against the experimental temperature decrease (Fig. 3b). However, some plots digressed at around 19°C; the plots appeared to regress on two lines (Fig. 4A and B). The correlation ratio was 0.94 in the partial range of 19–21°C while it was 0.41 in the range of 15–19°C.

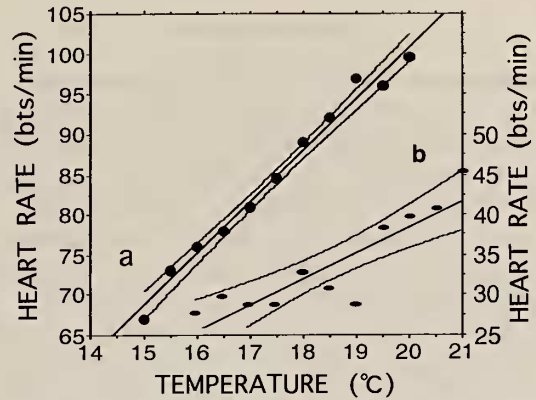


FIG. 3. Relationships between the heart rate and lowered temperature in the Japanese lobsters. The plots a, correlation between the mean heart rate and temperature measured during the period from spring to fall ($Y = 6.43X - 27.66$, $r = 0.99$). The plots b, correlation between the mean heart rate and temperature measured during winter ($Y = 3.26X - 26.38$, $r = 0.88$). A linear line and paired curves indicate the regression and 95% confidence bands for the true mean of the heart rate, respectively. The all data shown in Table 1 are used.

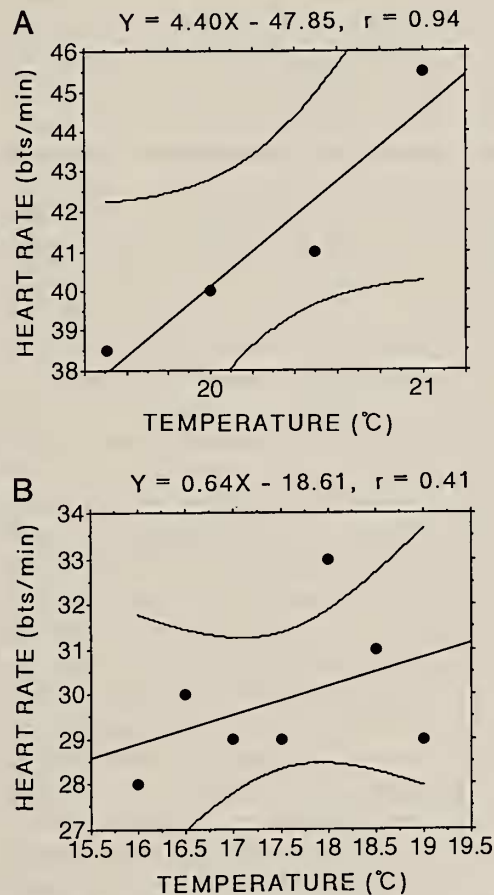


FIG. 4. The heart rate drop with cooling of the winter lobsters. A, the correlation between the mean heart rate and temperature in the range of 19.5–21°C. B, the correlation between the mean heart rate and temperature in the range of 15.5–19.5°C. A straight line and paired curves indicate the regression and 95% confidence bands for the true mean of the heart rate, respectively. The same data as shown in Fig. 3b are used.

DISCUSSION

The mean heart rate (45.5 bts/min) in winter was as low as half of that (99.5 bts/min) in the other seasons at the same acclimation temperature (Table 1). In *P. japonicus*, the heart rate around 60 bts/min is unstable both *in vivo* and *in vitro* [22]. In both the shrimp and the crayfish, the mean heart rate is lowered when reared under dark conditions [11, 23]. Since the lobsters used had been under natural light, they might perceive the decreases in both light dose and period toward winter. Therefore, the conditions of illumination may cause to lower the heart rate to 45.5 bts/min in winter lobsters. However, this idea is not acceptable at present because the experiments to prove it will be necessary.

The lobster heart in the pericardium is suspended by the ligaments and the arteries. The ligaments are associated with the pericardial alary muscles while the arteries with the pericardial septum, the circumference of which contains muscle fibers ([24] for review, [1]). These pericardial muscles are innervated by the segmental nerves coming from the thoracic ganglia [1]. Thus, the central nervous system (CNS) probably controls the heart rate *via* the suspensory elements mechanically. The importance of the suspensory elements for heart pumping activity has been noticed by several authors ([24, 25] for reviews, [13]). Meanwhile, we have observed that the beat rate of the isolated lobster heart is determined by pulling of the suspensory elements as well as filling pressure ([15], unpublished data). Therefore, the seasonal difference in heart rate levels *in vivo* might be determined by the degree of tension of the suspensory elements controlled by the pericardial muscles and the CNS.

The lobster heart *in vivo* is also controlled centrally *via* the dorsal nerves and the pericardial neurohemal organs [1–3, 26, 27]. The nervous control might contribute to the rapid change in heart rate observed frequently as fluctuations ([13, 14], Figs. 1A and 2A). The pericardial organs of lobsters contain monoamines (serotonin, octopamine and dopamine) and peptides (proctolin and FMRFamide-related peptides) ([6] for review, [12, 29, 30]). The pericardial hormones slowly augment the beat of isolated lobster heart for minute-long periods [5, 17, 18]. By internal cooling of the body, the pericardial organs of *P. japonicus* are activated and probably release the pericardial hormones [21]. Even under the low temperature, octopamine markedly enhanced the beat rate and amplitude of the isolated heart [20]. Therefore, octopamine released from the pericardial organs could activate the *in vivo* heart under the low temperature. The pericardial peptides also may activate the *in vivo* heart [31, 32] but their actions under low temperature have not yet been proved.

The cooling rate of 1°C/min is faster than that applied to decapod crustaceans by others [7, 10, 28]. Despite of the rapid cooling, the Q_{10} value for the lobster heart rate was around 2, similar to that reported in the crab [10]. Thus we failed to find the effect of rapid cooling on the Q_{10} value. When the water temperature was resumed, however, the heart rate was often higher than that before cooling. This

may suggest that the heart activation occurs during cooling and still continues during rewarming.

The Q_{10} between 10 and 25°C in the crustacean heart rate has been constant with an average value of about 2 while the Q_{10} of 3–5 has been often observed between 5 and 10°C ([24] for review). This indicates that the chemical pacemaking processes in the heart are enhanced under the cold conditions. The Q_{10} for the range of 15–20°C of the lobster heart rate was 2.6 in winter while it was 2.2 in summer. Moreover, the correlation between the heart rate and the lowered temperature was weaker in winter than in the other seasons (Fig. 3). Even if the starting heart rate was different, the decrease in heart rate against the same drop in temperature is much slow in winter (Fig. 4). It is therefore suggested that the heart activation for the cooling may occur markedly in winter.

The lobster cardiac muscle develops tension by excitatory junction potentials generated by motor neurons in the cardiac ganglion [4, 15, 19]. Tension produced in the myocardium is fed back to the cardiac ganglion since the cardiac neurons are sensitive for filling pressure ([24] for review, [15–18]). Thus, the responses of the heart to cooling should be explained by activities of the cardiac neurons and muscle fibers. The studies using the isolated hearts and their nerve-muscle preparations are in progress.

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REFERENCES

- Alexandrowicz JS (1932) The innervation of the heart of the crustacea. I. Decapoda. *Q J Microsc Sci* 75:181–249
- Alexandrowicz JS (1953) Nervous organs in the pericardial cavity of the decapod Crustacea. *J Mar Biol Ass UK* 31: 563–580
- Alexandrowicz JS, Carlisle DB (1953) Some experiments on the function of the pericardial organs in Crustacea. *J Mar Biol Ass UK* 32: 175–192
- Anderson M, Cooke IM (1971) Neural activation of the heart of the lobster *Homarus americanus*. *J Exp Biol* 55: 449–468
- Beltz BS, Kravitz EA (1986) Aminergic and peptidergic neuro-modulation in crustacea. *J Exp Biol* 124: 115–141
- Cooke IM, Sullivan RE (1982) Hormones and neurosecretion. In "THE BIOLOGY OF CRUSTACEA Vol. 3" Ed by HL Atwood, DC Sandeman. Academic Press, New York, pp 205–391
- Dauscher H, Flindt R (1969) Vergleichende Untersuchungen zur Herztätigkeit bei freibeweglichen dekapoden Krebsen (*Astacus fluviatilis* Fab., *Astacus leptodactylus* Escholz und *Cambarus affinis* Say.). *Z vergl* 62: 291–300
- Dudel J, Ruedel R (1968) Temperature dependency of electromechanical coupling in crayfish muscle fibres. *Pflüger Arch* 301: 16–30
- Fisher L, Florey E (1981) Temperature effects on neuromuscular transmission (opener muscle of crayfish, *Astacus leptodactylus*). *J Exp Biol* 94: 251–268

- 10 Florey E, Kriebel ME (1974) The effects of temperature, anoxia and sensory stimulation on the heart rate of unrestrained crabs. *Comp Biochem Physiol* 48A: 285–300
- 11 Hara J (1952) On the hormones regulating the frequency of the heart beat in the shrimp, *Paratya compressa*. *Annot Zool Jap* 25: 162–171
- 12 Kobierski LA, Beltz BS, Trimmer BA, Kravitz EA (1987) FMRFamide-like peptides of *Homarus americanus*: distribution, immunocytochemical mapping, and ultrastructural localization in terminal varicosities. *J Comp Neurol* 266: 1–15
- 13 Kuramoto T (1990) Cardiac activity and pressure change in the lateral pericardium of the unrestrained lobster, *Panulirus japonicus*. *Physiol Zool* 63: 182–190
- 14 Kuramoto T (1993) Cardiac activation and inhibition involved in molting behavior of a spiny lobster. *Experientia* 49: 682–685
- 15 Kuramoto T, Ebara A (1984) Effects of perfusion pressure on the isolated heart of the lobster, *Panulirus japonicus*. *J Exp Biol* 109: 121–140
- 16 Kuramoto T, Ebara A (1985) Effects of perfusion pressure on the bursting neurones in the intact or segmented cardiac ganglion of the lobster, *Panulirus japonicus*. *J Neurosci Res* 13: 569–580
- 17 Kuramoto T, Ebara A (1988) Combined effects of 5-hydroxytryptamine and filling pressure on the isolated heart of the lobster, *Panulirus japonicus*. *J Comp Physiol B* 158: 403–412
- 18 Kuramoto T, Ebara A (1991) Combined effects of octopamine and filling pressure on the isolated heart of the lobster, *Panulirus japonicus*. *J Comp Physiol B* 161: 339–347
- 19 Kuramoto T, Kuwasawa K (1980) Ganglionic activation of the myocardium of the lobster, *Panulirus japonicus*. *J Comp Physiol* 139: 67–76
- 20 Kuramoto T, Nakamura M (1992) Effects of cooling on the heart beat of the Japanese lobster *in vitro*. *Zool Sci* 9: 1217
- 21 Kuramoto T, Tani M (1994) Cooling-induced activation of the pericardial organs of the spiny lobster, *Panulirus japonicus*. *Biol Bull Mar Biol Lab Woods Hole* 186: 319–327
- 22 Kuramoto T, Yamagishi H (1990) Physiological anatomy, burst formation, and burst frequency of the cardiac ganglion of crustaceans. *Physiol. Zool*, 63: 102–116
- 23 Larimer JL, Tindel JR (1966) Sensory modifications of heart rate in crayfish. *Anim Behav* 14: 239–245
- 24 Maynard DM (1960) Circulation and heart function. In "The Physiology of CRUSTACEA Vol. 1" Ed by TH Waterman, Academic Press, New York, pp 161–214
- 25 McMahon BR, Wilkens JL (1983) Ventilation, perfusion, and oxygen uptake. In "THE BIOLOGY OF CRUSTACEA Vol 5" Ed by LH Mantel, Academic Press, New York, pp 290–372
- 26 Shimahara T (1969a) The inhibitory synaptic potential in the cardiac ganglion cell of the lobster, *Panulirus japonicus*. *Sci Rpt Tokyo Kyoiku Daigaku B14*: 9–26
- 27 Shimahara T (1969a) The effect of the acceleratory nerve on the electrical activity of the lobster cardiac ganglion. *Zool Mag* 78: 351–355
- 28 Spaargaren DH, Achituv Y (1977) On the heart rate response to rapid temperature changes in various marine and brackish water crustaceans. *Neth J Sea Res* 11: 107–117
- 29 Sullivan RE, Friend BJ, Barker DL (1977) Structure and function of spiny lobster ligamental nerve plexuses: evidence for synthesis, storage and secretion of biogenic amines. *J Neurobiol* 8: 581–605
- 30 Timmer BA, Kobierski LA, Kravitz EA (1987) Purification and characterization of FMRFamide-like immunoreactive substances from the lobster nervous system: isolation and sequence analysis of two closely related peptides. *J Comp Neurol* 266: 16–26
- 31 Wilkens JL, McMahon BR (1992) Intrinsic properties and extrinsic neurohormonal control of crab cardiac hemodynamics. *Experientia* 48: 827–833
- 32 Yazawa T, Kuwasawa K (1992) Intrinsic and extrinsic neural and neurohumoral control of the decapod heart. *Experientia* 48: 834–840

